

PHOTOOXIDATION AND TRIPLET FORMATION OF THE PRIMARY ELECTRON DONOR OF THE GREEN PHOTOSYNTHETIC BACTERIUM *PROSTHECOCHLORIS AESTUARII*, OBSERVED WITH ESR SPECTROSCOPY

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1. Introduction

Relatively little is known about the photochemical reaction in green photosynthetic bacteria. The high antenna bacteriochlorophyll to reaction center ratio of 1000–2000 BChl *c* + *a* per reaction center [1,2] and the difficulty of obtaining purified reaction center preparations has impeded the study of the electron transport by means of optical and ESR spectroscopy. In [3], we reported the isolation of photochemically active preparations of *Prosthecochloris aestuarii*. Starting from the membrane vesicle preparation complex I [2,4,5], the photosystem pigment (PP) complex and the reaction center pigment–protein (RCPP) complex were isolated, with antenna BChl *a* to reaction center ratios of ~75 and 35, respectively [3]. At room temperature both complexes show photooxidation of P-840, the primary electron donor, and of 1 cytochrome *c*-553/reaction center [3]. Upon flash excitation the formation of the triplet state of P-840 was observed with optical difference spectroscopy in both complexes [6]. At <120 K the amount of P-840 triplet formed in a saturating flash was 0.4–0.5 in the PP complex and close to 1/reaction center in the RCPP complex.

Here, we report on the kinetics of photooxidized P-840 and its triplet state monitored at 5 K with ESR and optical spectroscopy. Depending on the redox potential, the photooxidation of P-840 is reversible in the PP complex, yielding a dimer type BChl *a* free radical ESR signal at $g = 2.0025$. The triplet of P-840 is found to be spin polarized; the zero-field splitting parameters and the decay times of the different triplet levels are calculated. An average decay time of 280 μ s was found. This, and the value of the zero field param-

eters suggest that the BChl *a* dimer in green bacteria is structurally different from that in purple bacteria.

2. Materials and methods

Prosthecochloris aestuarii strain 2 K, was grown anaerobically in a mixed culture known as '*Chloropseudomonas ethylica*' [7] as in [8]. The membrane preparation complex I and the pigment–protein complexes PP and RCPP complex were prepared as in [3]. The light-induced absorbance changes were measured at 5 K with the apparatus described in [9], equipped with a xenon flash-tube (duration at half-maximum intensity, 13 μ s).

ESR experiments were performed with a Varian E-9 spectrometer, having an instrumental response time of 20 μ s, as in [10]. Continuous illumination with white light provided by a 1000 W projection lamp, filtered by 5 cm water and a Balzers Calflex C filter or xenon flashes (duration at half-maximum intensity, 5 μ s) were used as actinic illumination. The samples were contained in quartz tubes of 3 mm internal diameter. Before freezing to 5 K, the samples were frozen to 80 K in a nitrogen gas-flow cryostat. All samples contained glycerol (50%, v/v) to prevent crystallization on cooling.

3. Results and discussion

Fig.1 shows the ESR spectrum of the light-induced changes in the $g = 2$ region, obtained by illumination at 5 K of the PP complex prepared from *P. aestuarii*. The spectrum shows a free radical signal, symmetrically

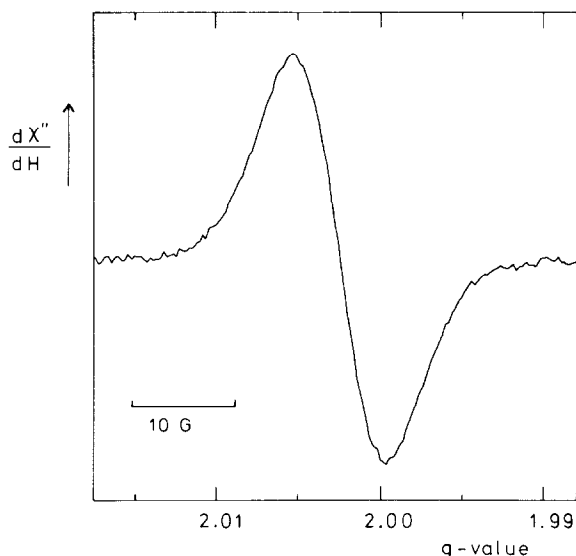


Fig.1. Light minus dark ESR difference spectrum obtained upon illumination in the $g = 2$ region at 5 K of the PP complex ($A_{810} = 2.1 \text{ mm}^{-1}$) at a redox potential of +70 mV: modulation amplitude 5 G; microwave power 0.2 mW; average of 4 scans.

centered at $g = 2.0025 \pm 0.0002$ with a peak-to-peak linewidth $\Delta H_{pp} = 9 \pm 0.2 \text{ G}$ that is due to photooxidized P-840, the primary electron donor. The g -value is indicative of a BChl radical, the linewidth indicates that P-840 is a dimer of BChl a , similar to the primary electron donor in purple bacteria. This is in agreement with measurements of P-840 photooxidation in complex I [11], a membrane vesicle preparation from *Chlorobium limicola* f. *thiosulfatophilum*. About 20% of P-840 appeared to be irreversibly oxidized at 5 K in the PP complex frozen at a redox potential of +70 mV. If the suspension contained in addition 100 μM PMS and 10 mM dithionite at pH 7.7, which poised the sample at -420 mV , the same light-induced ESR spectrum was obtained. In this case, however, P-840 oxidation was completely reversible. This indicates that at -420 mV forward electron transfer is blocked because of reduction of a secondary electron acceptor. This acceptor may be responsible for the complete irreversibility of P-840 oxidation in 'chromatophores' prepared from *C. limicola* f. *thiosulfatophilum* [12,13]. In [13] a partial reversal of P-840 oxidation in complex I of *C. limicola* was observed; however, lowering the redox potential to -590 mV did not eliminate the irreversible signal [13]. The

decay of the P-840⁺ signal at 5 K after a short flash had a half-time of 13 ms, probably due to the back reaction between P-840⁺ and a reduced acceptor, as observed at 77 K [6]. Flash-induced absorbance changes at 5 K, measured at 606 nm yielded the same half-time.

As was reported [6], only in $\sim 50\%$ of the reaction centers in the PP complex a stable charge separation is produced at $<120 \text{ K}$. In the other half, electron transport at the acceptor side appears to be inhibited, resulting in the formation of the BChl triplet of P-840, which decayed with a half-time of 165 μs at 120 K [6]. The ESR spectrum of the BChl a triplet, together with the P-840⁺ signal around $g = 2$, obtained upon illumination of the PP complex at 5 K is shown in fig.2. The AEEAAE polarization pattern, where A and E denote signals in absorption and emission, respectively, shows that the triplet is spin polarized by $S-T_0$ mixing, which is thought to be brought about by the radical pair mechanism [14,15]. Similar polarization patterns of the reaction center BChl a triplet were observed [16, 17] in cells and preparations of purple bacteria. The zero-field splitting parameters calculated from the triplet spectrum, $|D| = (207 \pm 2) \cdot 10^{-4} \text{ cm}^{-1}$ and $|E| = (37 \pm 1) \cdot 10^{-4} \text{ cm}^{-1}$ are, however, $\sim 15\%$ larger than those found for the reaction center BChl a triplet in a number of purple bacteria [18].

The decay of the triplet signal at the canonical x , y , z directions of P-840 was measured at 5 K at a microwave power of 2 μW . We assume that the number of transitions between T_0 and T_{\pm} , induced by this low level of microwave power is negligible during the

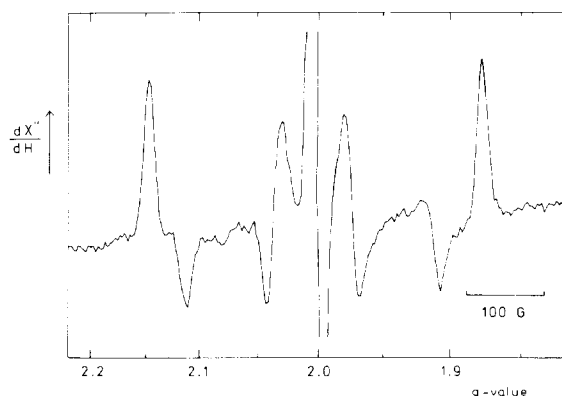


Fig.2. Light-induced ESR difference spectrum at 5 K obtained upon illumination of the PP complex ($A_{810} = 2.1 \text{ mm}^{-1}$) at a redox potential of +70 mV: modulation amplitude 20 G; microwave power 10 mW; average of 4 scans.

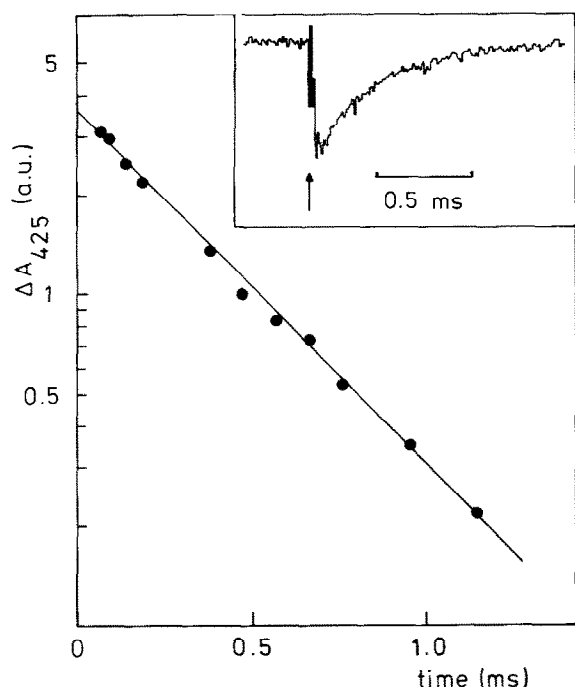


Fig.3. Absorbance change at 425 nm in the RCPP complex upon excitation at 5 K with infrared flashes provided by a combination of Schott RG 715 and RG 780 filters. The trace (inset) is the average of 128 flashes separated by 15 s. The semi-logarithmic analysis of the decay kinetics yields a half-time of 280 μ s.

lifetime of the triplet state. Therefore, the measured decay times of the different triplet levels represent the actual molecular decay rate constants k_x , k_y and k_z [19]. Rate constants of $1300 \pm 150 \text{ s}^{-1}$ for the z-component and $3000 \pm 400 \text{ s}^{-1}$ for the x- and y-component were measured under these conditions. These values for k_x and k_y differ considerably from those measured in purple bacteria [19–21].

Fig.3 shows the kinetics of the absorbance change at 425 nm, induced by a short flash in the RCPP complex at 5 K, which is ascribed to the triplet of P-840 [6]. Semilogarithmic analysis yielded a monoexponential decay within the limits of error, with a half-time of $\sim 280 \mu$ s, which is in good agreement with the average half-time ($290 \pm 30 \mu$ s) measured with ESR. In contrast to the ESR technique, where one can magneto-select the x, y and z directions, optical spectroscopy does not discriminate between the 3 triplet levels and the decay is a super position of the 3 decay channels. As the rates differ only by a factor of 2, a quasi-exponen-

tial decay with average half-time is observed. This situation contrasts with the optically measured low temperature decay of the triplet state of purple bacteria, where a biphasic decay is observed [22], due to the much larger spread in the rate constants.

The above results suggest that the relative orientation of the 2 BChl *a* molecules in P-840 in green bacteria differs from that in the primary electron donor of BChl *a* containing purple bacteria, resulting in a structurally different special pair [23]. This is also indicated by the difference in position of the maximal bleaching of the Q_y -bands (830–840 nm in green bacteria [2,5,6] and 870–890 nm in purple bacteria).

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